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# BIOLOGICAL BULLETIN

## ARTIFICIAL REMOVAL OF THE GREEN BODIES OF HYDRA VIRIDIS.

D. D. WHITNEY.

Whether the green bodies in hydras are infecting algæ or products of their own metabolism has been long disputed. I have discovered a method by which they may be removed and the hydras continue to live nevertheless and to multiply.

Semper suggested that these green bodies in *Hydra viridis* might be algæ. A little later Brandt made a careful study of them and concluded that they are algæ which probably live parasitically in the endoderm cells of the hydra.

At the same time Lankester also studied these green bodies. He said: "It appears to me that an examination of the green colored corpuscles of *Hydra* demonstrates those corpuscles to be similar in nature to the chlorophyll bodies of green plants, and that there is no more reason to regard them as symbiotic algæ than there is to regard the green corpuscles in the leaf of a buttercup as such."

Sallitt examined the green bodies in several species of Protozoa and found them to be identical with the green bodies of *Hydra* and *Spongilla*. This uniformity of the green bodies in different animals and their similarity to the chloroplasts of plants led to the belief on the part of a few zoölogists that the green bodies in animals and plants are identical and probably have the same function in both kinds of organisms.

Beyerinck in 1890 isolated the green bodies from *Hydra viridis* and succeeded in making pure cultures of them in an artificial medium. This demonstrated that the green bodies were different from the chloroplasts of plants. He, moreover, identified them with the alga, *Chlorella vulgaris*.

Various theories have been advanced to account for the presence and function of this green alga in animal cells. Some workers have maintained that these cells assimilate the $CO_2$ which is given off by the animal cells, and then in their turn give off oxygen which is used in the life processes of the animal cells. Others have imagined that the algæ might manufacture products in the presence of sunlight which are passed out into the animal cells and used by them as a food.

Instead of a translocation of the algal reserves to the animal tissues Famintzin and Beyerinck have shown that the alga itself is liable to absorption and digestion by the host. Gamble and Keeble found that mature and immature *Convoluta roscoffensis* digest masses of their own green cells and that the animals obtain little if any food by the translocation of the reserves of its green cells.

They also found that the alga is not transmitted through the egg to the following generation as in the case of *Hydra viridis*, but that the young embryos of each generation are infected by the alga as soon as they leave the egg. Furthermore, "the relation between animal and green cells is a complex one, and cannot be described as symbiotic. The green cell once in the body of the animal probably never escapes; either it is digested or it dies when the animal dies."

In the winter of 1905–6 while keeping *Hydra viridis* in various chemical solutions in order to find some means of causing the development of the reproductive organs it was discovered that animals kept in a weak solution of glycerine lose their green color.

A series of experiments was carried on at that time and in the following winter and spring under the direction of Prof. T. H. Morgan and Prof. W. J. Gies. The following data will show the nature of the results obtained:

*Experiment I.*— February 19, 1906. Temperature 20°C. Several green hydras were put into a 1.25 per cent. solution of glycerine without food.

February 26. Only 3 were alive, and appeared white to the ordinary eye. These were placed in spring water without food.

March 3. The 3 hydras were beginning to become green in color at the oral end.

March 23. Hydras had normal green color.

*Experiment II.*— March 1. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine.

March 1. One hydra which appeared white to the eye, but which showed a single green patch of algæ in one tentacle under the lens, was isolated in spring water without food.

March 18. Green color had redeveloped gradually and was at this time identical with that of an ordinary hydra.

*Experiment IV.*— April 16. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine without food.

April 30. Four hydras which showed no green color under the lens were isolated in spring water without food.

May 7. The hydras showed no trace of green color under the lens. Experiment discontinued.

*Experiment VIII.* — May 5. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine. Fed every 72 hours with rotifers, *Hydatina senta.* Several formed buds in the glycerine solution.

May 24. Nine hydras which showed no trace of green color under the lens were isolated in a .25 per cent. solution of glycerine and the feeding continued. One individual had a bud attached which itself was budding. None of the others had buds.

May 26. One of the other 8 individuals was budding.

May 27. Three of the 8 individuals were budding.

May 29. Buds had become detached from two of the hydras. Experiment discontinued.

*Experiment X.* — March 12, 1907. Temperature 20° C. Many green hydras were put into a .5 per cent. solution of glycerine without food.

March 27. Began to add food every 24 hours.

April 7. Isolated 30 hydras which showed no trace of green color under the lens in small glasses containing spring water.

April 23. None had budded. Some had died and some were developing green color.

April 25. Fifteen hydras alive. Nine showed no trace of green color under lens and six showed green patches of algæ scattered in various parts of the body.

Lot A. April 25. Put three of the white hydras into a large balanced aquarium. Food added every 24 hours.

April 27. The three hydras were larger and each had one bud attached.

April 28. The buds had become detached from parent hydras.

April 30. The buds were larger than when detached. Each of the 3 parent individuals was budding again.

May 1. One of the parent hydras had two buds attached.

May 2. Nine individuals. Two of the parent hydras were budding. None showed a trace of green color under the lens.

May 5. Eleven individuals.

May 8. Twelve individuals, 5 of which were budding.

May 10. Sixteen individuals, 4 of which were budding, one had 2 buds. None showed a trace of green color under the lens.

May 27. Many white hydras on lighted side of aquarium.

Lot B. April 27. Put 6 white hydras into another large balanced aquarium which contained green hydras. The white hydras were not in very good condition ; they were fed daily with rotifers.

May 1. Only two white hydras alive.

May 13. Several white hydras seen on walls of aquarium in the midst of the green ones. Some individuals were budding.

May 27. Many white hydras on the lighted side of aquarium.

*Experiment XII.*—April 4. Put one *Hydra fusca* together with many *Hydra viridis* into a .5 per cent. solution of glycerine without food.

April 14. Food added.

May 1. Twelve of the *Hydra viridis* had lost all green color. *Hydra fusca* was reddish orange in color. It had produced several buds.

May 8. Eight *Hydra fusca*, three of which were budded. Much larger and very different in color from the *Hydra viridis* that had lost their green color.

May 13. Thirteen *Hydra fusca*. Two individuals had 1 bud, 3 had 2 buds and 2 had 3 buds attached.

*Experiment XIII.* — April 14. Put several green hydras into a .5 per cent. glycerine solution. Food added daily.

May 10. Some hydras had no visible green color and were rather small in size.

Lot A. May 10. Put 5 of the white hydras into spring water without food.

May 20. All alive and white. Four were on the lighted side of the jar.

May 27. Five hydras alive. Two showed no green color but 3 had small green patches around the oral end.

Lot B. May 10. Put 6 of the white hydras into spring water without food.

May 20. All alive and white. Three were on lighted side of jar and 3 were on the bottom of the jar.

May 27. Four hydras alive. One showed no green color but 3 had small green patches around the oral end.

The action of the glycerine upon the green hydra is to cause the algal cells to leave the entoderm cells and to pass into the digestive cavity, from which they are expelled to the outside through the mouth when the hydra contracts. In many instances when hydras were examined which had been in the glycerine solution for a few days, masses of the green algæ could be seen in their digestive cavities. In one case the expulsion of the algæ was actually seen. In other cases no masses of the algæ were ever seen. However, the bottom of the glass upon which the animals were usually located always became more or less green within a small radius of each individual, showing that the algæ were expelled and sank to the bottom of the dish. The algæ never increased to any noticeable extent, but apparently died.

It seems evident from these experiments and observations that the alga in *Hydra viridis* does not play a very important rôle in the life processes. The animal is able to live many days in the glycerine solution without food while in the process of losing its algæ. In Experiments IV. and X. the hydras were without food for about 14 days, and in other experiments the animals have appeared to be in a normal condition, except smaller in size, when kept in glycerine solution without food for 21–30 days. Experiments IV. and XIII. also show that hydras that have lost all their green color can live at least for 7–17 days in spring water without food and at the end of this time be in a normal condition. Very likely they can live a much longer time than this without food.

The loss of the algæ does not seem to interfere with the process of bud formation in the slightest degree. Experiments VIII. and X. show that if the white hydras are fed sufficiently they will produce buds at a normal rate and in a normal manner.

It is a well-known fact that green hydras will live several weeks without food but of many hydras that I have kept 2–5 weeks in various experiments without food none ever produced buds, thus showing that the alga does not furnish food enough for bud formation. However, if sufficient food is given to either the white or green hydras buds soon appear.

The hydras from which all algæ have been extracted do not become reinfected with the algæ. In Experiment X. both lots of the white hydras were kept in large balanced aquaria in which there were hundreds of green hydras, but they remained white. Furthermore the white hydras were fed upon rotifers, the digestive tract of which was usually filled with *Euglena viridis*. It will be recalled that Sallitt believed that the green bodies of *Euglena* were identical with those of the green hydra. However, the colorless hydras are not infected by even this contaminated food supply.

The reappearance of the algæ in some of the supposed white hydras can be readily explained by the supposition that all of the algæ were not removed from the endoderm cells of the hydras before they were transferred to spring water. When the animals were placed again in their normal environments the alga began to grow and reproduce itself until the hydras became as green as normal ones.

The white hydras seems to respond to the stimulus of light in the same manner as the green hydras. In Experiment XIII., where the hydras were starved, some of them collected on the lighted side of the dish and the others remained upon the bottom. None were found on the least illuminated side of the dish.

In Experiment X., lot A, the white hydras were suspended in the large aquarium in a small dish. They did not leave the dish, but climbed upon its lighted side. The food, *Hydatina senta*, shows no reaction to light, so that it cannot be supposed that the hydras moved towards a food supply.

Some of the white hydras of lot B in the same experiment left the

small dish, which was suspended in another large aquarium about three inches from the lighted side, made their way to the lighted side and there became attached. Those that remained in the small dish collected upon its lighted side.

Some of the early workers suggested that *Hydra viridis* might be a variety of *Hydra fusca* which had acquired the power of harboring green algæ in its cells. Greenwood and others found that the endoderm cells of green hydras contain brownish colored bodies similar to or identical with the same colored bodies in the cells of *Hydra fusca*. This brownish color is masked in *Hydra viridis* by the green color of the algæ.

Interesting as this suggestion is, the evidence from the colorless hydras is opposed to it. The *Hydra viridis* that has lost its green color in the glycerine solution is smaller in size, has shorter tentacles, produces fewer buds at any one time than *Hydra fusca*. It has a very faint tint of pink or brown color while *Hydra fusca* which has been in a glycerine solution 2–5 weeks has a reddish orange color.

## Summary.

1. When *Hydra viridis* is kept in a .5–1.5 per cent. solution of glycerine it loses all its green color.

2. The green alga passes out of the endoderm cells into the digestive cavity from which it is expelled through the mouth to the outside when the hydra contracts.

3. The green alga does not continue to live outside in the glycerine solution.

4. The white hydras will live at least seventeen days without food.

5. The white hydras if fed can produce buds at the same rate and in the same manner as green hydras and have been kept alive for more than two months.

6. The white hydras do not show a trace of green color, under the lens, after seven weeks feeding upon contaminated food. These hydras were kept in spring water during the first 2–3 weeks of feeding and then placed in a large balanced aquaria which contained many hundreds of green hydras and much algæ. Thus showing that the white hydras were not reinfected under very favorable conditions.

7. The white hydra is positive heliotropic like the green hydra in moderate illumination.

8. The white hydra does not resemble *Hydra fusca* but, except in color, retains all the specific characters of *Hydra viridis* from which it is derived.

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# A NOTE ON THE ADAPTIVE SIGNIFICANCE OF THE SPERM-HEAD IN CEREBRATULUS.

N. YATSU.

While studying the fertilization processes in the living eggs of *Cerebratulus lacteus* at South Harpswell, Me., I was struck by the fact that it took the spermatozoa considerable time and not a little effort to bore through the thick membranes in order to reach the egg. From this I concluded that the long, slender and slightly curved head of the spermatozoön of *Cerebratulus lacteus* might have evolved in correlation with the thick egg-membrane characteristic of this species (Fig. 1, *A*).[1]

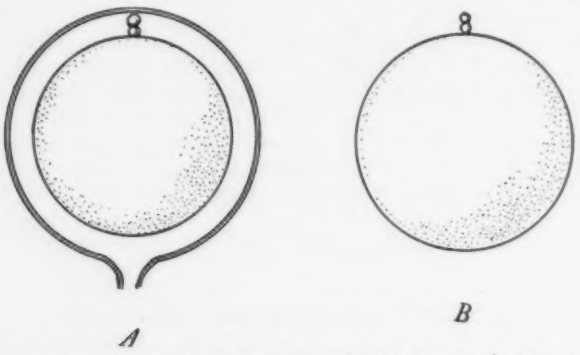


FIG. 1. Egg of *Cerebratulus lacteus*, (*A*), and of *C. marginatus* (*B*). × 220.

Since the study of sections was begun this conclusion has been strengthened. The spermatid has a round head. Later the anterior portion of the head is gradually drawn out into a slender beak, the head proper still remaining pear-shaped. At the last stage of this transformation the beak thickens and the head proper elongates. Thus the typical shape of the sperm-head is attained (Fig. 2, *A*). After entering the egg, the sperm-head repeats in reversed order the process just described, finally giving rise to a

[1] The membrane is made up of two layers. By accident spermatozoa sometimes find their way into the space between them.

round sperm-nucleus, which does not differ much from that of the spermatid.

When informed by Professor E. B. Wilson that the egg of *Cerebratulus marginatus* has no membrane (Fig. 1, *B*), I thought that if the above conclusion were true, this species must have a spermatozoön with a blunt head. At Naples in the spring of 1906, I found that this expectation was fulfilled. Instead of the slender pointed head found in *Cerebratulus lacteus* (10.6 $\mu$), the Neapolitan form (*C. marginatus*) has a spermatozoön with a blunt head (5.4 $\mu$) terminating in a knob as shown in Fig. 2, *B*. The length of the tail is nearly the same in both forms.

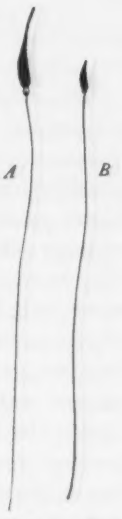


FIG. 2. Spermatozoön of *Cerebratulus lacteus* (*A*), and of *C. marginatus* (*B*). × 1133.

The difference in size of the sperm-heads might be interpreted as due to the number of chromosomes contained in them. In fact *C. lacteus* has 18 or 19 chromosomes in the reduced number, while *C. marginatus* has 16 according to Coe.[1] Yet the difference in shape of the sperm-head between two such closely allied forms as these is difficult to explain without taking into consideration a special adaptation for boring thick membranes (cf. Pflüger and Smith, '83[2]). This, I think, is an actual instance to support the general belief that the diversity in the shape of sperm-head has evolved in response to the mechanical needs for penetrating the egg.

ZOÖLOGICAL LABORATORY,
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NEW YORK, June 8, 1907.

[1] Coe, W. R., '99, "The Maturation and Fertilization of the Egg of *Cerebratulus*." *Zool. Jahrb. Abth. Anat. u. Ont.*, 12.

[2] Pflüger, E., und Smith, W. H., '83, "Untersuchungen über Bastardierung der anuren Batracier und die Principien der Zeugung." *Pflüger's Archiv*, 32.

# ON PARTHENOGENESIS IN SPIDERS.[1]

THOS. H. MONTGOMERY, JR.

The only references known to me upon the question of the occurrence of parthenogenesis in araneads are the following: Blackwall (1845) took young females of *Tegenaria domestica*, *T. civilis*, *Agelena labyrinthica*, *Ciniflo atrox*, *Drassus sericeus*, *Theridion quadripunctatum* and *Segestria senoculata* and kept "most of these individuals . . . in captivity from one to three years after they had completed their moulting and attained maturity; yet three only, an *Agalena labyrinthica*, a *Tegenaria domestica*, and a *Tegenaria civilis*, produced eggs, and they proved to be sterile, though several of the others, to which adult males were subsequently introduced, laid prolific eggs after coition." Blanchard (1857) also reached the same conclusion that eggs laid by unimpregnated females prove sterile. Then Balbiani (1873) adopted this conclusion, though his own observations were not decisive: "Having imprisoned during a whole year several females of *Tegenaria domestica*, I have noted that the first batches were composed exclusively of fecund eggs, while the subsequent batches contained always a variable number of sterile eggs, of which the quantity increased with the batch, so that they ended in not containing a single egg able to develop. But it is evident that if these females had been gifted with the faculty of reproducing without the concourse of the male, all the successive batches should have been equally fecund."

On the other hand, Campbell (1883) kept a female of *Tegenaria guyonii* in captivity a whole year, during which she underwent two moults; then she laid eggs from which young hatched. And Damin (1893) imprisoned a female *Filistata testacea* Latr. from the spring of 1891 until the spring of 1893; she moulted twice in the summer of 1891 and once in the spring of 1892, then made a cocoon from which young spiders emerged. He notes the extreme rarity of the males of this species, and asks: 'Does not this absence of the male indeed indirectly cause the,

[1] Contributions from the Zoölogical Laboratory of the University of Texas, no. 86.

parthenogenesis of *Filistata*?" It may be remarked that *Artemia salina* is an instance of this kind. A paper by Holmberg (1878) has been inaccessible to me.

It is generally held that the females of spiders are not ready for coition until they have passed their final moult, and that not until then does the copulatory plate, the epigynum, become fully developed. However, Bertkau (1885) has shown that *Atypus piceus* oviposits several years in succession, and a moult occurs (with change of the seminal receptacles) after the first years of egg-laying; he remarks that the same is probably true also of *Gnaphosa lucifuga*. This would show that spiders may undergo moults after they are fully mature, and indeed it is very likely that when the female lives several years, and this is known to be the case in a number of species, she undergoes a moult each year after reaching maturity, for moulting is a necessary integral part of the excretory process. Then I (1903) have described the case of a *Lycosa bilineata* (Emerton) (*L. ocreata pulchra* Montg.) that copulated successfully on June 3, and on the following July 12 moulted. During the present year I caught a *Filistata* with a cocoon containing young; she moulted on July 2 and again on August 28. These instances indicate that spiders may be sexually mature before their final moults.

But one can be sure that a female is immature when her epigynum is still a small, smooth plate, and that when she is in such a condition she cannot be impregnated. And during the course of earlier observations on the mating habits I have noted that males avoid females that are not mature. Accordingly, females of Entelogynæ found with their epigyna small and imperfect may be considered virginal.

During the spring of this year the common *Lycosa relucens* Montg. was found in large numbers in the early part of March, males and females running over the ground in a wood at Austin, Texas. At that time very few mature individuals of either sex were discovered, the greater number being one or two moults removed from the mature condition. When they become full grown they are rarely found running upon the ground in the daylight, but then usually remain hidden under leaves and stones. Twenty-two females were secured, some on the third and the

others on the ninth of March, and kept isolated in glass cases, with fairly rich feeding, until June 12. All of these when caught showed the epigyna small and imperfect and this fact, in connection with the observation that few of the males at that time of the year were mature, made it certain that all the females were virginal. All underwent moults during captivity, one moulted twice, the others only once; after moulting all but four made cocoons containing eggs. Eleven of the spiders made one cocoon each, seven made two cocoons each, and one made three cocoons. Of the total of twenty-eight cocoons, eleven were destroyed by the spiders shortly after their construction, the mothers eating the eggs, and most of these cocoons were very imperfect; while the remaining seventeen were removed from the mothers immediately after their completion to save them from such possible demolition, handled as gently as possible, and kept in separate bottles to test their fecundity. But not one of the eggs in any of these seventeen cocoons hatched, nor even reached the stage of the early blastoderm; one batch of eggs was fixed at the age of twenty-four hours, but on examination showed no cleavage nuclei near the surface, so they had certainly not reached the sixteen-cell stage. All these eggs were shrivelled and dry.

Therefore virginal females of *Lycosa relucens* form cocoons with eggs in them, but these eggs do not develop. And such females show always more or less imperfect construction of the cocoons and the tendency to eat the eggs; in mature individuals of *Lycosa* that I have bred in captivity, the eggs always developed, and the mother rarely ate the eggs.

My observations corroborate those of Blackwall and Blanchard, and in the species watched by us normal parthenogenesis seems not to occur; on the other hand, there are the two positive cases of its occurrence mentioned by Campbell and Damin. Certainly parthenogenesis is exceptional in spiders.

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# RHYTHMICAL ACTIVITY IN INFUSORIA.

S. J. HOLMES.

While much in the behavior of the infusoria comes under the head of direct responses to external stimuli, there is, in many forms, an extraordinary amount of activity which cannot be traced to any outside cause. In addition to those forms which keep up a continuous swimming with never-flagging energy, there are several infusorians which perform movements of a more or less regular rhythm. These are analogous to such rhythmical movements as the beating of the heart of higher animals, or the rhythmical pulsations of the swimming of a jelly-fish. They are more automatic than the latter, and are, perhaps, more closely comparable to the regular pulsations, which, under certain conditions, are performed by some species of jelly-fish after removal of the nerve ring and marginal sense organs.

More or less regular and apparently spontaneous movements have been noted in a few species (*Stentor*, *Vorticella*) by various writers, but the subject has received scarcely more than a passing mention. My attention was called to this feature of the behavior of certain infusoria in some studies recently made on the behavior of *Loxophyllum meleagris*, and I was led to look for similar phenomena in other forms. Observations were made on the following species:

*Loxophyllum meleagris.* — *Loxophyllum* commonly moves about on some solid object by extending the body, gliding forward a short distance, then swimming backward, turning toward the oral side and then going forward again. The changes in the direction of movement are not due to any obstacles encountered; they occur in much the same way when there are no objects in its course. The organism frequently keeps up this kind of movement for a long time in very nearly the same locality. The body is always narrowed in swimming forward, and always widened in swimming backward, thus showing a constant correlation of the contractile activities with the direction of the ciliary beat. If the body is cut in two, the pieces will undergo the same rhyth-

mical back-and-forth movements. Even very small pieces, less than one sixteenth of the body, show the same regular rhythm and the same correlation of ciliary and contractile activity.

*Dileptus gigas.* — *Dileptus gigas* commonly adheres to the surface of some solid object and waves its long proboscis-like anterior extremity or neck about in an anti-clockwise direction. The surface of the body is quite sticky, as is shown by the fact that it adheres readily to any object brought in contact with it. The slender extremity in its movement about in a circle executes many twists and curls in more or less irregular ways. These movements may be very vigorous or they may be very slow, but they scarcely ever entirely cease. The slender neck is very extensile and may be elongated to three or more times its length when in a contracted state.

*Dileptus* often executes short forward and backward movements at tolerably regular intervals. During its movement forward the body elongates, and while gliding backward it widens, showing the same correlation of contractility with the direction of the beat of the cilia that occurs in *Loxophyllum meleagris*. The backward and forward excursions vary exceedingly in length. Frequently they are exceedingly short. Even when the organism remains attached in one place the body undergoes more or less regular elongations and contractions while waving about the anterior extremity. There is a rhythm here much as in the preceding species occurring quite independently of external stimulation. The posterior third of the body when severed from the rest still undergoes elongations and contractions, although in a somewhat lessened degree. In larger pieces the rhythm of movement is more manifest.

*Lachrymaria olor.* — This interesting species resembles *Dileptus gigas* in its general behavior as well as its external form. Its long flexible neck is kept continually waving about, but the extensions and contractions of its body do not occur so regularly as in the preceding species.

*Vorticella.* — *Vorticella* frequently shows quite regular rhythmic contractions without an apparent external cause. The peristome with its membranellæ is folded in and the stalk contracts into a spiral form. In a short time the spiral straightens out, the peri-

stome expands, and another contraction soon follows. Hodge and Acking found that the interval between successive contractions in *Vorticella* varied greatly. In one individual kept for a long time under continuous observation contractions occurred at one time about once in four seconds, at another once in eight seconds, and at various other intervals in different times. Sometimes there was no rhythmic contraction at all. The stimulus to the rhythmic contraction of the stalk apparently comes from the body, for the stalks which I have isolated showed no independent movements.

*Stentor.* — *Stentor cæruleus* when attached and extended sways about slowly in a circle. The swaying is a very regular movement and is not due to any evident external stimulus. It is a result of the contraction of the body instead of the action of cilia, as the stalk is bent successively in different directions. There is also a rhythmic movement executed by *Stentor roeselii* during the construction of its tube. This species after attaching itself alternately contracts and extends its body in a more or less regular manner while it is secreting the gelatinous substance of which its tube is formed. The swaying movements are not so pronounced as in the preceding species.

It is a noteworthy fact that rhythmical activities occur in those species which are either attached like *Stentor* and *Vorticella*, or which like *Loxophyllum*, *Dileptus* and *Lachrymaria* frequently remain for a long time near one spot. These forms do not have to wait for something to turn up, but are actively seeking for new stimulations, their rhythmical movements bringing them in a measure the advantages which in forms like *Paramœcium* are secured by almost continuous swimming.

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# FURTHER STUDIES ON THE PARTHENOGENETIC DEVELOPMENT OF THE STARFISH EGG.

D. H. TENNENT.

The results of investigations described in an earlier paper[1] led me to the view that possibly a conjugation of egg and sperm chromosomes, similar to that apparently occurring in starfish eggs that had been treated with $CO_2$ and subsequently fertilized, might be found to occur in normally fertilized eggs.

As I suggested in the paper mentioned, it would be necessary, in order to settle the question raised, to reëxamine the normal fertilization and cleavage stages or to make a study of the formation of the germ cells in the starfish.

This paper deals with observations made in accordance with this plan and with some further observations made on starfish eggs. developing as a result of treatment with $CO_2$. The material for the investigation was obtained while I was occupying a room at the Marine Biological Laboratory, Woods Hole, during a portion of the summer of 1906.

I found, soon after beginning a study of fertilized starfish eggs. that the equatorial plate of the first cleavage spindle contained, with variations which I shall mention later, in eggs from some individuals 18 chromosomes, and in eggs from other individuals 36 chomosomes. I have as yet been unable to correlate this difference in the number of chromosomes with the common starfishes of the Woods Hole region, *Asterias forbesii* and *Asterias vulgaris*,[2] although it is probable that such a relationship will be established.

The study of the fertilized eggs proved puzzling, and it was not until I had made an investigation of the spermatogenesis of *Asterias vulgaris* and a reëxamination of eggs developing parthenogenetically after treatment with $CO_2$ that I was able to find a solution for the problem under consideration.

[1] "Studies on the Development of the Starfish Egg," D. H. Tennent and M. J. Hogue, *Journal of Experimental Zoölogy*, Vol. III. (1906).

[2] Clark, "The Echinoderms of the Woods Hole Region," Bull. U. S. F. C., Vol. XXII. (1902), pp. 553-554.

Inasmuch as the basis of my interpretation lies in facts observed during the study of the male germ cells, I shall first present a brief account of these observations.

## The Spermatogenesis of Asterias vulgaris.

In well-preserved stronger Flemming material stained in iron-hæmatoxylin the spermatogonia show 18 chromosomes, these all having a slightly constricted or dumb-bell form (Fig. 1). The chromosomes are either straight or slightly bent.

The chromosomes of the primary spermatocytes are nine in number and have at first a distinct dumb-bell form. A precocious longitudinal splitting soon gives them a V or looped form which may be seen in horizontal sections of the equatorial plate (Fig. 2).

1 2 3 4 5

Fig. 1. Equatorial plate of spermatogonial mitosis.
Fig. 2. Spermatocyte of the 1st order.
Fig. 3. Spermatocyte of the 2d order. Polar view.
Fig. 4. Spermatocyte of the 2d order. Metaphase.
Fig. 5. Second spermatocyte division.

The secondary spermatocytes contain nine chromosomes (Figs. 3 and 4). In the second maturation mitosis these appear to be divided transversely (Fig. 5), giving nine as the reduced germ-cell number.

## Studies on Eggs.

After noting the difference in the somatic number of chromosomes in the different lots of eggs sectioned, it became evident that it was desirable to have a set of material in which the eggs from one individual had been treated in three different ways, namely, one set fertilized with sperm, another set treated with $CO_2$ and a third set treated with $CO_2$ and subsequently fertilized. I succeeded in obtaining one lot of material of this nature. I shall record my observations in the order of the above statement.

### (*a*) *Observations on Fertilized Eggs.*

In a successful preparation, the section being sufficiently thick to show the greater number of the chromosomes of the equa-

torial plate of the first segmentation spindle in their entirety (Fig. 6), the chromosomes are seen to be of a dumb-bell form, some straight, some slightly bent, and lying with their long axis placed transversely to the spindle fibers. Their number, as may be seen from the figure, would lead one to suspect 36 as the somatic number, but owing to the fact that some of the chromosomes are cut, this may not be stated with certainty.

In an especially fortunate section passing symmetrically through the long axis of the spindle, it is seen that the chromosomes have been split longitudinally and drawn out as somewhat slender rods. In drawing this figure I have shown only the chromosomes and parts of chromosomes lying within a short

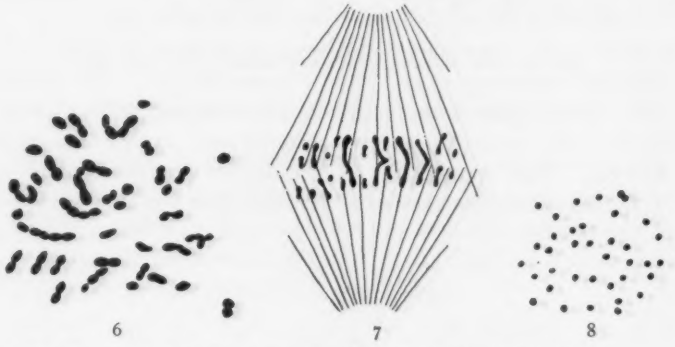


FIG. 6. Equatorial plate 1st segmentation fertilized egg. Polár view.
FIG. 7. First segmentation fertilized egg. Anaphase.
FIG. 8. First segmentation fertilized egg. Section through chromosomes as they are drawn out in anaphase.

focal range, inasmuch as it would have complicated the figure so greatly as to make it unintelligible had the chromosomes lying toward the opposite side of the spindle been added. In this section again, the full number of chromosomes could not be counted with certainty.

Finally, in a section passing transversely through the spindle of an egg in the same stage of division as that from which Fig. 7 was drawn, it is shown conclusively that the somatic number of chromosomes in this lot of fertilized eggs is 36.

### (*b*) *Observations on Eggs Treated with* $CO_2$.

Due precautions were of course taken to avoid chance fertilization. The control showed freedom from segmenting eggs.

In the sections of these eggs it was even more difficult than in the fertilized eggs to determine accurately the number of chromosomes. Sections thick enough to contain all of the chromosomes were unintelligible. Thinner sections were likewise of

FIG. 9. *a–b*. Sections through same equatorial plate $CO_2$ egg.

little value. Fig. 9 shows all of the chromatic material contained in the equatorial plate as demonstrated in two sections of this egg. The impossibility of stating with any reasonable degree of accuracy the number of chromosomes involved is evident

FIG. 10. *a–d*. Four longitudinal sections through 1st segmentation spindle, late anaphase. $CO_2$ egg. Spindle fibers omitted.

to any observer. Nor is the situation appreciably relieved by the examination of longitudinal sections of the spindle in later anaphase.

In such sections as those shown in Fig. 10, *a–d*, a cursory

examination would lead the observer to believe that the number of chromosomes is fully as great as that in the fertilized eggs. This view might be supported by the fact that many of the chromosomes show a form similar to that possessed by those of the fertilized eggs.

Closer examination of the position and arrangement of the chromosomes in these sections reveals the fact that the bodies have been sectioned. The imaginary superposition of one figure upon the other lends credence to such an idea.

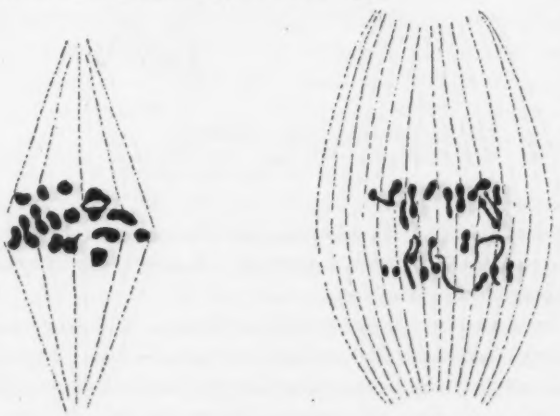
For these reasons I have been unable to determine the number of chromosomes by actual count. The number I believe to be 18, a statement for which I shall give my reasons later.

### (*c*) *Eggs Treated with $CO_2$ and Subsequently Fertilized.*

Sections of these eggs agree with figures that I have already published. The eggs were fertilized and underwent segmentation, the $CO_2$ simply retarding the rate of development.

### Observations on Other Starfish Eggs Treated with $CO_2$.

I succeeded in obtaining one lot of eggs which developed after treatment with $CO_2$, that contain in all cases only 9 chromosomes. I was unable to obtain a ripe male at the time and so can give no facts as to the fertilization of these eggs.



11 12

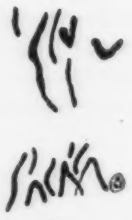
Fig. 11. First segmentation $CO_2$ egg. Early anaphase.
Fig. 12. First segmentation $CO_2$ egg. Later anaphase.

The individual from which I obtained the eggs I identified as *Asterias forbesii* although it will be noted that the number of chromosomes agrees with the germ-cell number in my spermatogenesis material of *Asterias vulgaris*.

In these eggs, as in the other $CO_2$ eggs described, the form of the chromosomes is irregular but owing to the smaller number may be counted readily. The equatorial plate, the daughter plates, etc., all show the same number, — *i. e.*, nine (Figs. 11 and 12).

Fig. 13 shows the extremely irregular form assumed by the chromatic material in anaphase and explains the reasons for the complexity exhibited by sections such as those from which Fig. 10 was drawn.

13



14

FIG. 13. First segmentation $CO_2$ egg. Anaphase.

FIG. 14. First segmentation $CO_2$ egg. Late anaphase. One chromosomal vesicle.

As shown in Fig. 13 this chromatic material at this stage is in the form of greatly twisted threads. A single section may cut the thread in several places.

In later anaphase these threads are drawn out, passing through a variety of changes and at last are embodied in chromosomal vesicles which unite to form the daughter nucleus (Fig. 14).

Clearly then, this egg with its oöcyte number of 9 chromosomes does not exhibit the phenomenon of "autoregulation."

As I have already stated, owing to my inability to make an accurate count, I have not been able to show this to be true in the case of the egg whose reduced number of chromosomes is 18. That the behavior of both eggs is probably similar will be granted by most readers.

## General Considerations.

These observations show conclusively that in the fertilized egg there is no conjugation of maternal and paternal chromosomes as individuals, at the time when I thought that such a union might take place.

The facts that I have given, namely, that the reduced number of chromosomes in the male germ cell of one form is 9, that the reduced number of chromosomes in one egg is 18, and that the number in fertilized eggs is 18 and 36 is sufficient proof.

After the examination of many lots of fertilized eggs I became convinced that 18 and 36 were not constant as somatic numbers. Small variations, such as differences of one or two, might be laid to error in counting. A constant greater variation that I have found hardly seems due to the same cause.

A possible interpretation of such a greater variation is of interest.

In one lot of eggs the number 27 seems constant. In this lot I have never been able to count as many as 36 chromosomes.

Such a number, (27), is readily explained on the supposition that an egg containing 18 chromosomes has been fertilized by a spermatozoan containing 9, or that an egg with 9 has been fertilized by a spermatozoan containing 18. The result in either case would be a somatic number of 27.

Now, accepting the interpretation of synapsis as the conjugation of homologous maternal and paternal chromosomes, we shall have at the conclusion of synapsis a reduced number of 18. That is, nine pairs or nine bivalant chromosomes and nine univalents which had been unable to find mates.

Such eggs, if they were fertilized by a spermatozoan containing 18 chromosomes should give rise to individuals with a somatic number of 36, or, uniting with a spermatozoan with 9 chromosomes should retain a somatic number of 27.

If the theory of the individuality of the chromosomes is correct and the interpretation of synapsis well founded, experiments in hybridization with favorable forms ought to prove the truth of such an explanation. The starfish, owing to our inability to raise adults from the egg and to the extremely small size of its chromosomes, does not seem promising for such an investigation.

## Summary.

1. The reduced number of chromosomes in the male germ cells of *Asterias vulgaris* is 9.

2. Fertilized starfish eggs contain as a somatic number 18 and 36 chromosomes, the difference possibly to be correlated with *Asterias vulgaris* and *Asterias forbesii*.

3. Eggs caused to develop parthenogenetically show one half the somatic number of chromosomes.

4. No conjugation of individual chromosomes takes place in fertilized eggs immediately before the first segmentation.

5. A possibly hybrid form contains 27 chromosomes.

Bryn Mawr College,
July, 1907.

All of the figures are from camera drawings made with aid of Zeiss No. 12 compensating ocular and 2 mm. apochromatic objective. Some of the sketches were subsequently doubled in diameter by means of a drawing camera. These have been reduced one half in reproduction. The others are reproduced as drawn.

# THE REACTIONS OF THE BLIND FISH, AMBLYOPSIS SPELÆUS, TO LIGHT.[1]

FERNANDUS PAYNE.

## Introduction.

In the Woods Hole Biological Lectures ('99) Dr. Eigenmann gave the results of some experiments to determine the reaction of *Amblyopsis* to light. He recorded that:

1. *Amblyopsis* seeks the dark regardless of the direction of the rays.

2. An individual coming from a dark chamber into a lighted one shows signs of uneasiness.

3. A light ray thrown on fishes from a mirror causes uneasiness in from one to five seconds.

4. Bright sunlight causes the fishes to swim uneasily.

5. A lighted match held above an aquarium, which had been in the dark, caused two fishes in one instance to dart to the bottom. In another case it produced a very general and active movement among forty individuals.

6. Different colors do not cause different reactions.

7. In an open pool, the fishes remained under rocks during the bright part of the day.

It is the purpose of the present paper to repeat some of his experiments, but in a different way; to add others, to give data in full of how the fishes react and to determine why they react. The special interest in the problem lies in the fact that we are dealing with a blind animal, whose remote ancestors possessed well-developed eyes.

Part of the work was done at the Indiana University cave farm at Mitchell, Indiana; the remainder at the university. All the material used was caught in the caves at Mitchell.

These fishes are very sensitive to mechanical stimuli and with this in mind every possible precaution has been used to eliminate them. At Mitchell the cave was used as a laboratory and the

[1]Contributions from the Zoölogical Laboratory of Indiana University, No. 89.

aquarium placed on the solid earth, so no better nor more natural conditions could have been found. At the university, the experiments were made in a basement dark-room with black walls, where the temperature remained practically constant. The aquarium was set on a stone pedestal.

Only very simple experiments were made and conclusions have not been reached until after an examination of more than one series of fishes, although the data of only one series will be given.

## Apparatus.

My apparatus consisted of an aquarium, a light screen, a heat screen and a lamp. The aquarium was 18 inches long, 15 inches high and 12 inches wide. The light screen was made of heavy cardboard and was placed between the end of the aquarium and the heat screen, so as to shut out all light except that entering from one end of the aquarium. A small aquarium was used for a heat screen. Its sides were of clear glass, parallel and placed 3 inches apart. This screen was placed 2¼ inches from the end of the aquarium. The water used in both the aquarium and heat screen was filtered. Two lamps were used, one an acetylene lamp of one hundred candle power, and the other an arc lamp of eight hundred candle power.

## Experiments and Observations.

My experiments at Mitchell were made with the hundred candle power lamp. First, the lamp was placed 32 inches from the end of the aquarium and with it in this position the whole aquarium was lighted. As the aquarium was 18 inches in length there was considerable difference in the intensity of the light at the two ends. With ten fishes, ranging from 2½ to 4 inches in length in the aquarium, counts were taken once a minute for thirty minutes, and at the end of this time the counts showed 163 fishes in the end of less, and 137 in the end of greater intensity. Before making this observation and with the aquarium lighted just enough to enable me to see the fishes as white objects, I took counts to see whether they showed any preference for one end of the aquarium over the other. Thirty-five counts gave me 176 fishes in one end and 174 in the other. So the large number of

fishes in the end of greater intensity could not have been due to a preference for that end.

With the light in the same position and with half of the aquarium darkened by means of a light screen, counts were again taken as before. First one half of the aquarium and then the other was made dark. Sometimes these counts showed more fishes in the dark; at others more in the light. Under natural conditions the fishes swim very slowly. During these observations their movements were much faster, thus indicating that they are photodynamic. All subsequent observations, whether the light was of a low or high intensity, brought out this fact very distinctly.

On account of the shape of the lamp, it could not be brought closer than 32 inches and still illuminate the whole aquarium. I therefore made a small aquarium, 7 inches long, 5 inches high and 4 inches wide, and suspended it within the larger. In this case the heat screen was removed as there was always three or four inches of water between the fishes and the lamp. With the lamp only nine inches from the end of the small aquarium and with half of the aquarium darkened in various ways, I took a large number of counts. These observations were made on three series of fishes. The results were again conflicting. In the majority of cases a larger, sometimes a much larger number of fishes were seen in the dark, but sometimes a larger number were observed in the light. The fishes seem to be disturbed more with the light at this distance than when it is 32 inches from the end of the aquarium.

At the university an 800 candle power arc lamp was used. The heat and light screens were again placed in position and during the observations the entire aquarium, except the end where the light entered, was covered with heavy black cloth. The fishes were transferred from the cave to the aquarium in a closed vessel and hence were not exposed to the light. They were left in the aquarium, at least twenty-four hours before observations were made, so they could become accustomed to the new conditions. Also after taking one series of counts they were left several hours before making other observations. With the lamp 16 inches from the end of the aquarium, counts were taken every

minute for thirty minutes and with ten fishes in the aquarium. First the right half was darkened. These counts gave me 84 fishes in the light to 216 in the dark. I then lighted the whole aquarium to see whether they would seek the end away from the light, *i. e.*, the end of less intensity. These counts gave me 85 fishes in the end of greater and 215 in the end of less intensity. Here is a difference of 130, while with the one hundred candle power lamp 32 inches from the end of the aquarium, there was a difference of only 26.

With the aquarium just sufficiently light for me to see the fishes, I took counts to see whether they remained at the surface or near the bottom. These counts showed 101 fishes in the upper half and 199 in the lower. This indicates that they are positively geotropic. To determine whether their positive geotropism would be overcome by their negative heliotropism, I darkened first the lower and then the upper half of the aquarium. With the lower half dark, the counts showed 56 fishes in the light to 244 in the dark and with the upper half dark 121 in the light to 179 in the dark. While their geotropic reaction is partly overcome by their negative heliotropism, it is not wholly so.

To determine whether the direction of the rays of light plays any part in the movements of the fishes, the light was placed 14 inches above the surface of the water. As no convenient heat screen was at hand, a clear glass which fitted snugly against all sides of the aquarium, was lowered 2½ inches beneath the surface. Thus there was always 2½ inches of water between the fishes and the light. With the lamp in this position, I darkened first one end and then the other and took counts as before. Thirty counts with the left end dark showed 68 fishes in the light and 232 in the dark, and with the right end dark 64 in the light and 236 in the dark. At the end of each count, I shifted the light screen to the opposite end, and each time the fishes within two or three minutes changed to the dark end again. This immediate change, with the shifting of the light, proves conclusively that the light is the only factor which caused them to seek the dark. These counts confirm the conclusion of Eigenmann that they seek the dark regardless of the direction of the rays.

With the light coming from above, we get a larger percentage

of fishes in the dark than when the light strikes them from the side. It is possible that with the light overhead, the brain and spinal cord are affected directly on account of the transparency of the tissue above them.

For comparison, I caught a number of small specimens ranging from 15 to 25 mm. in length, placed 10 of them in the aquarium and made observations as I had done with the adults. In these fishes, the eye was plainly visible as a small black spot beneath the skin, while in the adults there is no external indication of an eye. With the light at the end of the aquarium and the whole aquarium lighted, the thirty counts showed 115 fishes in the end of greater and 185 in the end of lesser intensity, as compared with 85 to 215 in the case of the adults. With the left half dark there were 60 in the light to 240 in the dark, as compared with 98 to 202 in the adults. With the right half dark, 48 in the light to 252 in the dark, against 84 to 216 adults. The light was then placed above the aquarium as before and counts taken. When the left end was dark there were 28 fishes in the light to 272 in the dark and with the right half dark there were 25 in the light to 275 in the dark, as compared with 68 to 232 and 64 to 236 in the case of the adults. Here again we have a larger percentage of fishes in the dark when the light is above, and, further, these young fishes seem to be more sensitive to the light than the adults. What is the reason for this? Thinking that the eye might play some part in this difference I removed the eyes from 10 young. Seven of these recovered in good condition. Of these I took five, all of which were about an inch in length, and 52 hours after the operation made the first observation.

With the light at the end and the left half dark, thirty counts gave me 19 fishes in the light to 131 in the dark, and with the right half dark, 22 in the light to 128 in the dark, as compared with 48 to 252 and 60 to 240 in the case of the young with eyes. These observations show that there is practically no difference in the reactions of the young with eyes and those without eyes. Hence the eyes play no part in the reaction.

In making these experiments the counts often varied considerably in the same series even though the external conditions, so

far as I was able to determine, were exactly the same. However, this is to be expected, since the fish is a highly complex organism and its internal mechanism is not the same at any two times.

To determine whether the skin is equally sensitive on all parts of the body, I used a light focused to a point by means of a Zeiss *a* * objective. With the aquarium dark, I focused this light on various parts of the body. Sometimes they reacted and sometimes they did not, but they reacted as often with the light focused on the tail as on the head, and *vice versa*. Later I placed some fishes in a dark corner of a room and with a mirror threw sunbeams on various parts of the body. In nearly every case I got a definite reaction. Sometimes the fishes turned around and swam in the opposite direction and sometimes darted forward. Further, they reacted as often when the light was thrown on the tail as on the head. Judging from these experiments they are equally sensitive on all parts of the body. However, this is what we might expect since all parts of the skin are exposed to like conditions. Parker ('05) concludes that the tail of Ammocœtes is most sensitive to light, but he accounts for this by the fact that Ammocœtes burrows head foremost into the sand.

Eigenmann ('99) states: "Two examples [of blind fishes] kept in a pail in my cellar were quietly floating, but when a lighted match was held above them, the fishes at once darted to the bottom and sides of the pail." This is not a common reaction. I have tried the lighted match again and again and also have flashed the one hundred candle power lamp above them, and in no case did they dart to the sides or bottom immediately. In fact, they did not react immediately when the eight hundred candle power lamp was flashed on them. With the fishes in their native habitat I have made a number of observations with the one hundred candle power lamp by flashing it upon them as they lay perfectly quiet in the water, and in each case it was from 10 to 30 seconds before any movement took place. In nearly all cases the movement was either to one side or straight ahead and not toward the bottom. My results are more in accord with his observation on forty individuals when a lighted match "produced a very general and active movement among all individuals."

In the same paper Eigenmann records the action of a colony of Amblyopsis in an open pool. "During the bright part of the day, the fishes always remain under the rocks at the bottom. In the morning and evening and at night they could be seen swimming about in various parts of the pool." At Mitchell, near the entrance of one of the caves, is a small pool, the bottom of which is covered with rocks. I found two fishes in this pool. They were probably washed there during times of high water, as the water runs from one cave to the other at such times. Later, I put two more fishes into the pool and as I was making daily trips to the cave I often noticed them swimming about near the surface. The pool was not in the direct sunlight but the sun reached it, in patches, between twelve and one o'clock, and I took a number of observations at this time. I watched the pool for 15 minutes at a time and out of 13 observations made on 13 different days was able to see from one to three fishes ten times out of the thirteen. Sometimes they came out only to go immediately back under the rocks, but they often remained at the surface from five to ten minutes. Apparently this seems to conflict with my former experiments, but such is not the case, because under no condition did the fishes remain in the dark all the time. I do not mean to say that in the pool the fishes remain in the light more than in the dark, but that they do come out at times even in the brightest part of the day.

## Conclusions.

1. *Amblyopsis* is negatively phototropic.
2. The young are more sensitive to light than the adults.
3. The young deprived of eyes are as sensitive as those with eyes. Hence the eyes play no part in their reactions.
4. They seek the dark regardless of the direction of the rays.
5. When stimulated with a light focused to a point they seem to be equally sensitive on all parts of the body.
6. They are positively geotropic.
7. They are photodynamic.
8. These fishes are sensitive to light of low intensity and this sensitiveness increases as the intensity of the light increases.

# THE ANTENNÆ OF DIPTERA; A STUDY IN PHYLOGENY.

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CHICAGO, ILL.

No classification of the order Diptera is, on the whole, satisfactory. Four or five more or less elaborate schemes have been proposed by various writers in the past, but none has received general endorsement by students of the order. Scarcely any two writers agree as to the relationships of the larger part of the families, nor as to the value of many of their distinguishing characters. And this unsatisfactory condition is doubtless largely due to our failure to differentiate between homoplastic or convergent resemblances and genetic characters. As in all other large groups of animals there have been many phyletic lines of descent, many parallel adaptations to like environments, and these adaptive characters, here as elsewhere, have been, too often, used as fundamental classificational characters. Of past writers Osten Sacken was, I believe, most appreciative of such accidental resemblances; but even he often mistook adaptive for hereditary characters I am convinced.

The attainment of like characters by evolution by no means necessarily implies common ancestry. One would not think of uniting all flies having two-jointed palpi in one group, nor all those having a club-shaped abdomen in one suborder. But there are many other characters, less conspicuous ones, which have been used for such purposes, whose origins have been due to adaptations; and it will be long before we have thoroughly learned to distinguish them. New characters acquired in different phyla are seldom, perhaps never, exactly alike, though there may often exist the most curious resemblances, due doubtless to similar determining causes, or to orthogenesis, if there be such a thing. To paleontology we are indebted for the formulation, at least, of the apparent law that evolution is irreversible — that organs once functionally lost are never regained. There may be exceptions to this rule, but, so far, the history of past life seems

to teach that there are not. A fly with two-jointed palpi, for instance, could not have been ancestral to one with four joints in these organs.

Of all the organs of diptera, the antennæ, it seems to me, have received less critical comparative study than any others, and I believe that there is a fertile field here for fruitful phylogenetic studies. The differentiation between the nematocerous and brachycerous flies was, for a long while, based almost exclusively upon the structure of these organs; until it was conclusively shown that, by themselves, they have little classificational value. The antennæ, for instance, of *Bibio* are so nearly identical with those of *Xylophagus* that they might be interchanged without affecting generic characters, even as the wings and palpi of the phorids might be interchanged with some of the scatopsines without affecting generic characters. It was doubtless because of this primary division long ago by Latreille and Macquart into "many-jointed" and "three-jointed" antennæ that a misconception still exists among many as to the real structure of these organs. There are very few three-jointed antennæ among diptera, and even these will usually show, under high magnification, vestiges of additional joints. The great majority of existing diptera have five or six joints in their antennæ, and this majority includes the whole of the Cyclorrhapha, with but few exceptions. The fallacy has been in considering the antennal style or "arista" as an outgrowth or addition to the real antenna, whereas it is of course merely the specialized and more or less attenuated distal (?) part of the flagellum, showing all stages of attenuation and abbreviation; and it is yet to be shown that the arista is quite homologous in all diptera.

In the following table I have condensed the results of considerable observation and research on the structure of the antennæ in the different families of flies. It is of course impossible for one to examine critically all the genera of diptera, and the published data are yet, in many cases, inexact, and this inexactness is especially apparent when it comes to the detection of vestigial or minute joints. I have found under critical examination not a few instances of minute joints which have been neglected by systematists in general. Such a study as the present one must

take into account all vestiges and aborted organs if one would arrive at precise results. The Phoridæ, for instance, have been shown by Brues and others to possess two scape joints, though but one has usually been ascribed to them. And this will probably be found to be true of all the other so-called two-jointed antennæ, such as those of certain cyrtids, empidids and dolichopodids, under close examination. I have arranged the families in the following list not quite in the supposed order of their relationships, in order to bring out more clearly the antennal structure. I have also for the few families showing archaic forms given the extremes in parenthesis, with the "normal" or usual numbers in the regular column.

| | | | |
|---|---|---|---|
| Tipulidæ (6–39) | 12–16 | Acanthomeridæ | 10 |
| Cecidomyidæ (6–36) | 12–16 | Tabanidæ | 6–10 |
| Psychodidæ | 12–16 | Leptinæ | 3–8 |
| Mycetophilidæ | 12–16 | Nemestrinidæ | 5–6 |
| Pachyneurinæ | 12–16 | Mydaidæ | 4–5 |
| Rhyphidæ | 12–16 | Apioceridæ | 3–5 |
| Dixidæ | 15 | Asilidæ | 3–5 |
| Culicidæ | 14–15 | Therevidæ | 3–5 |
| Blepharoceridæ | 9–15 | Scenopinidæ | 3 |
| Chiromomidæ | 6–15 | Bombyliidæ | 3–5 |
| Orphnephilidæ | 11–12 | Dolichopodidæ | 4–5 |
| Bibioninæ | 8–12 | Empididæ | 3–5 |
| Scatopsinæ | 9–10 | Lonchopteridæ | 6 |
| Simuliidæ | 10 | Phoridæ | 6 |
| Xylophaginæ (13–30) | 9–10 | Cyclorrhapha | 5–6 |
| Stratiomyidæ | 7–10 | | |

In this list we are at once struck with the predominance of five groups having the maximum normal number of sixteen, fifteen, ten, six and five. And I venture to suggest that these five groups represent, in the main, long since divergent phyla of diptera. Not invariably of course, because coincidences may and often do occur in the different lines of descent. There are quite a number, it is seen, having the maximum number of fifteen. Possibly these may represent one common branch from the sixteen-jointed antennæ, possibly several. But, in none of these groups, unless it be the fifteen jointed, and of course the primitive Tipulidæ and

Cecidomyidæ, do I believe that we shall often, if ever, find vestigial joints additional to the maximum — simply for the reason that the loss of additional joints has been so far back in geological history that vestiges have wholly disappeared.

If the law of irreversibility in evolution be true, then it is apparent that no fly has regained the use of a joint of the antennæ once functionally lost. The question at once becomes important: What was the original number of antennal joints in the Diptera? If we could only be assured of the origin of the order from the main insect stem, we might, perhaps, answer this question with satisfaction. But, since we cannot we are forced to depend upon the internal evidence presented by the diptera themselves. May we assume that this primitive number was sixteen, the number so conspicuous in the table? Or was it thirty-nine (or more) a number known in a single species of diptera? I have assumed that the evolution of the dipterous antenna has been by the reduction of the number of segments, and never by accretion. And I believe that this assumption is justified, though the matter is perhaps open to debate. There are very few forms of diptera known possessing more than sixteen antennal joints. Some species of *Pachyrhina* and of the nearly allied *Nephrotoma* among the Tipulidæ have nineteen joints in the male, fifteen or sixteen in the female. The genus *Ctenonia*, of the same family, from Chile, has twenty-two or twenty-four joints in the flagellum of the females of two species, fifteen in that of a third. . . . The very closely related *Cerozodia*, from Australia and New Zealand, with two species, known only in the males, has thirty-two and thirty-seven flagellar joints respectively, the largest number hitherto discovered in any dipteron. As Osten Sacken truly said: "The close affinity between *Cerozodia* and *Ctenonia* affords a new instance of the curious relationships between the Australian and New Zealand fauna and that of Chile [South America]; a relationship exemplified in abnormal forms, apparent survivals of past ages, of which we already have" many other equally remarkable instances in all branches of animal life, recent and fossil. And precisely similar is the relationship between *Tanyderus pictus*, of the same family, from Chile, with twenty-five antennal joints, and *T. ornatissimus* from Amboina, with twenty-two joints.

*Gynoplistia*, another tipulid genus, from Australia, New Zealand, New Guinea and Celebes, with numerous species, has from sixteen to twenty antennal joints, branched like those of *Cerozodia*. Are these forms really survivals of primitive types? I do not think that we are permitted to doubt it. Their habitats and distribution alone indicate that, and the fact that three of the few known forms of diptera with multiarticulate antennæ are known only from the Miocene is also corroborative. *Magachile*, perhaps identical with *Protoplasa*, is one of these amber forms.

Among the Cecidomyidæ we have a few forms with multiarticulate antennae, as many as thirty-six in the males of some *Hormomyiæ* with a maximum of twenty-four in the females. The only other forms with abnormal multiarticulate antennæ that I can discover in the literature, are *Rhachicerus*, with from twenty to thirty joints, *Chrysthemis*, an amber genus with twenty-three joints; and *Electra*, also from the amber, with thirteen joints, all belonging to the xylophagid "Brachycera," having a normal maximum number of ten joints. The fact that some of these examples have a larger number of joints in the male antenna than in the female, may seem to indicate that the increased number is an acquired secondary sexual character, and that the female antenna is nearer the primitive number. But, why may we not assume that the diminished number in the female is the real acquired sexual character, and not the increased number in the male? Certainly this *must* be the case with such forms as *Tanypus* and its allies among the Chironomidæ, *Micromyza* and others of the Cecidomyidæ. From the frequent occurrence of sexual variation in these apparently primitive forms, I think it is probable that the early diptera all had fewer antennal joints in the females than in the males. I am confident that we are safe in accepting at least thirty-nine as the original and primitive antennal number among diptera; safe in the belief that the evolution of the dipterous antenna has always been by reduction from this primitive number, and never by the reacquirement of joints once lost.

Just how the loss of antennal joints has occurred is not always clear. It may be assumed that it has been by the loss of distal segments, but this is certainly not always the case, especially when

these distal joints have been highly specialized. The scape is perhaps never entirely reduced. The genus *Chionea*, a wingless tipulid, has the conical third antennal joint terminating in a slender, three-jointed style, a structure very much like that of the Nemestrinidæ, for instance. Do these joints represent the first four of the flagellum? Osten Sacken thinks that the reduced number of twelve joints in *Toxorhina*, belonging in a group having the normal number of sixteen, is due to the coalescence of the basal joints of the flagellum. The stratiomyid genus *Chrysochlora*, as one of numerous instances, with the normal number of flagellar joints, has the last or eighth specialized into a slender arista. Is this arista homologous with the arista of the housefly, for instance, where it is serially the fifth or sixth? The defect of the Comstock-Needham system of venation nomenclature is the assumption that the disappearance of veins has always been due to coalescence, whereas we positively know that in many cases it has been due to their loss without coalescence. Has the reduction of the flagellum been the result of the close fusion of segments, or the absolute loss of proximal ones; or has the arista been variously and repeatedly produced by the attenuation of the last segment or segments, whichever they happen to be? I believe that the arista has usually resulted from the former method and that it generally is homologous. It is quite clear, however, that the diminution in the number of homologous or homonymous joints has often been due to the loss of distal segments, whatever may have been the case with heteronymous forms; and the proof of this is apparent in the oftentimes vestigial condition of the terminal joints. The subject, however, is one worthy of investigation, and may throw light on the relationships of many of the diptera.

Sixteen antennal joints seem to be the primitive normal number of the modern Nemocera, a number acquired so long ago that very few examples yet remain of the more primitive condition. Do they indicate a single phylum? It seems doubtful. The forms with multiarticulate antennæ not only belong in the three chief subdivisions of the Tipulidæ, but are also found among the Cecidomyidæ, which would seem to indicate that the number sixteen had been acquired independently in different lines of de-

scent — that family or subfamily differentiation had occurred before the final reduction took place. It is also curious to observe that the minimum number of antennal joints in the Tipulidæ, Cecidomyidæ and Chiromomidæ is six, precisely the maximum number of the Cyclorrhapha.

In living forms the maximum and very common number of flagellar joints among the Brachycera, with the exception of *Rhachicerus*, is eight, found so frequently in the Xylophagidæ, Stratiomyidæ, Tabanidæ and Acanthomeridæ. Is this coincidence of phylogenetic significance? I feel quite sure that it is. I think that no one can dispute the relationships between *Rhachicerus* and the Xylophaginæ. Is *Rhachicerus* a belated survival of the xylophagid ancestors? If so, the phylum must have branched off long ago from the Tipulidæ (the venation excludes all other families, save the Rhyphidæ), before the antennæ had become reduced below thirty segments. One thing at least seems very evident, the Rhyphidæ are not the nearest related to the Xylophagidæ of the nematocerous families, as is usually believed. The Brachycera had their origin evidently directly from the ancestral Tipulidæ.

Likewise all of the five-jointed families would seem to be excluded from ancestral relationship with the six-jointed forms, and especially the Cyclorrhapha, though possibly the reduction has occurred since divergence.

While the antennæ, taken separately, may offer suggestions as to phylogenies of the dipterous families, and while they may absolutely veto such theories as imply reversion, they can settle none by themselves; they must be correlated with all the other organs of the body, and must harmonize with theories derived from other organs. I offer, nevertheless, the foregoing suggestions, in the possibility or probability that they may find corroboration

Secondary sexual characters are transmitted by heredity to the other sex, unless inhibited by sexual utility, or, possibly, sexual selection. It was for the casual statement of this law to a class in paleontology that I have recently been made the victim of a sensational press. The primitive eyes of diptera were doubtless separated by the front equally in both sexes. As a sexual character the eyes have become contiguous above the antennæ,

almost invariably a male character. There is evidently a sexual use for this greater development of the eyes in the male that has preserved the character with but little tendency to transmission to the female. It has, however, been transmitted to the female in some instances, and there are a very few forms in which the female has acquired the character in advance of the male. Examples of the former may be found among the Cyrtidæ, but a better one is that of *Systropus* of the Bombyliidæ, with contiguous eyes in both sexes, while the very nearly related *Dolichomyia* has the female eyes separated by the front. It was for the contiguity of the eyes in the male that Osten Sacken twenty-five years ago proposed the convenient term "holoptic," the antithesis of which, "dichoptic," was suggested by me a little later. But Osten Sacken's meaning of the term has been somewhat misunderstood. He gives as a definition of his Nemocera vera the nonholopticism of the eyes, while it is well known that some forms in this group do have contiguous eyes. But Osten Sacken really meant sexual holopticism, not simply contiguity of the eyes on the front. It remains to be proven that sexual holopticism does not really occur among these families of flies. If so, however, the occurrence must be extremely rare.

The primitive dipteron must have had eight fully developed longitudinal veins (including the auxiliary vein), with the second, third, fourth and fifth furcate; and a complete discal cell. The head was rather small, with the compound eyes separated equally by the front in both sexes. The ocelli were functional, and the maxillary palpi had four freely articulated joints; the labial palpi had probably already disappeared, though Wesche thinks differently. There were at least thirty-nine antennal joints in the male. The prothorax, mesothorax and metathorax were imperfectly fused, and the metanotum was visible from above. The abdomen had nine functional segments; the body was without differentiated bristles; and the tarsi had membranous pulvilli and empodia. The primitive flies were of moderate or small size, and probably crepuscular in habit, or at least denizens of shady forests.

Of modern diptera the Tipulidæ approach most nearly this hypothetical ancestor, but they have become specialized by a general increase in size, by the almost complete loss of the

ocelli; by the loss of the pulvilli; and the loss of the branch of the third vein, save among some of the Ptychopterinæ. The Rhyphidæ come next, but they have acquired holoptic eyes in some forms. Of the other families, the Psychodidæ and Culicidæ are perhaps the nearest allied to the original type, notwithstanding the occasional occurrence of multiarticulate antennæ among the Cecidomyidæ. The Culicidæ evidently represent an old type geologically, recrudescent in later times. Their fixed venation and antennal structure could only have come from long inheritance in a phylum which has not yet reached decadence. The blood-sucking habit of the mosquitoes is doubtless a rather recently acquired one, probably since the great development of the warm-blooded animals, as is evidenced by the almost innumerable sexual modifications of the palpi, modifications seldom found among the other families of Nemocera. The mosquitoes doubtless arose from the Corethrinæ, now decidedly on the wane. Every family, save the Tipulidæ, is I believe, absolutely excluded from immediate genetic relations with the Brachycera, because of the venation and the antennæ. I am, upon the whole, inclined to the belief that Osten Sacken was right in insisting upon the taxonomic importance of the Nemocera, as one of the chief phyla of diptera.

# DO ANTS FORM PRACTICAL JUDGMENTS?

C. H. TURNER.

Scattered through the literature are several records of observations which indicate that ants form what Hobhouse calls practical judgments. However, recent comparative psychologists favor the casting of such evidence out of court, because it was not obtained from experiments conducted under proper conditions of control. Lubbock[1] experimented upon the subject with negative results. The first two bits of evidence reported in this communication are reports of mere observations; the only excuses for recording them are that the observations were made in the laboratory and that they confirm the records of similar observations made by others in the field; the third, however, is the result of a carefully planned and controlled series of experiments.

For several months I have had in one of the laboratories of the University of Chicago a colony of *Camponotus herculeanoligniperdus*, consisting of nine winged females and about twice as many workers.[2] The Janet nest in which the ants were housed contained a row of three compartments (Figs. 1 and 2; *A*, *B*, *C*). The entrance to this nest (Fig. 1, *E*) was about one centimeter wide by two centimeters high. For several weeks no obstructions of any sort were placed by the ants in that entrance, although a large amount of litter was kept upon the island at all times. An ant usually mounted guard in the entrance *E* and similar guards were usually stationed in the tunnels connecting compartment *C* with compartment *B* and compartment *B* with compartment *A*. For over two months I examined the nest several times daily and in over ninety per cent. of the times I found guards located in the places mentioned.

In the course of some experiments upon an entirely different problem I had occasion to fight the guard stationed in the entrance

[1] Lubbock, "Ants, Bees and Wasps," London.

[2] To prevent the experiments recorded in this paper from being invalidated by mice, all holes leading into the room were filled with plaster of paris and the door was kept locked whenever I was absent.

*E* (Fig. 1) with dissecting needles and glass rods. Sometimes I used plain needles or glass rods, at others needles or rods that had been moistened with oil of cedar or oil of cloves. Each time the fight was continued until the ant retreated into the innermost part of the nest. After these maneuvers had been repeated several times daily for about a week, the guard withdrew from the entrance *E* and the ants plugged that passage-way with detritus, composed of bits of wood and bread from the island, trash from the interior

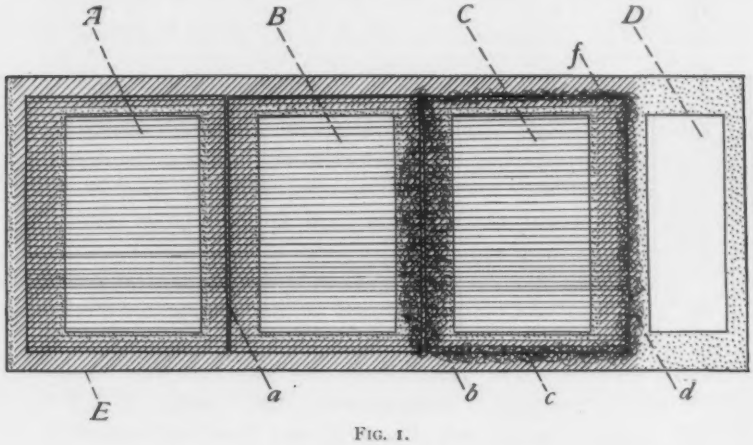


FIG. 1.

of the nest and cotton stripped from the turkish toweling of the nest. For five months thereafter the nest was examined from five to ten times every day, but no ant was observed as a guard, in either entrance *E* or the tunnels connecting the apartments until August 10, 1907. Since then they have been mounting guard as regularly as they did before they plugged the entrance.

On the same Lubbock island with the above-mentioned *Camponotus-herculeano-ligniperdus*, I had a large colony of *Formica fusca* var. *subsericea* Say. The island was kept littered with detritus of various kinds. One day I noticed a worker of this colony begin the construction of a bridge across the ditch of water that surrounded the island on which the colony was located. This partial bridge was constructed in the following manner. The ant placed a piece of charred paper about one centimeter wide

upon the water on the inner side of the ditch. After walking out upon this bit of paper and reaching outward with its antennæ, the ant returned to the island and picked up a crumb of bread crust three millimeters wide. The ant then walked across the charred paper and placed this bit of bread upon the water just beyond and adjacent to the paper. After standing for a short time upon the outer edge of the crumb and reaching outward with its antennæ, the ant returned to the island and picked up a piece of wood two millimeters wide by three millimeters long. This the ant placed upon the water just beyond and adjacent to the crumb. Thus there was constructed, extending three fourths of the distance across the ditch, a bridge of three elements. Upon the outer terminus of this partial bridge the ant stood for fully two minutes, reaching continually outward with its antennæ. The bridge was never completed.

The third bit of evidence was afforded by a series of experiments performed upon the same colony of *Formica fusca*. This colony, consisting of three wingless perfect females and about two hundred workers, was brought into the laboratory September 27, 1906. It was from the sidewalk of one of the streets of Chicago, where it was located partly beneath the stone and partly in the trash that the ants had heaped up along the edge of the stone. The sidewalk, which was several years old, was composed of concrete stones at least a yard square and fully six inches thick. The stones of this particular pavement were free from cracks of all kinds. In the laboratory the ants were kept in a nest of the type described above. Compartments *C* and *B* were covered with orange glass and compartment *A* with colorless glass. Throughout all of the experiments the Lubbock island upon which the nest was located was kept littered with bread crumbs, bits of wood, small pieces of egg shell, partially burned matches, charred paper, cotton, etc. For nearly three months the ants of the colony were the subjects of daily experiments upon the sense of hearing, etc. After that, for several weeks, the ants were left to themselves, but the nest was carefully scrutinized several times every day. At all times some workers would be found in apartment *B*, but the fertile females and the majority of the workers used compartment *C* (Fig. 1) as a living room. Whatever booty the ants captured was always carried into this chamber.

On the twenty-sixth of December, 1906, I discovered three batches of newly laid eggs in the living chamber. For several days prior to this discovery, an ant from the nest had been busy covering crack *b* (Fig. 1.) with detritus obtained from the island. At first only one ant was thus occupied. Later in the day a second ant joined this one. On some days three and on others four ants were thus engaged. These ants worked on for about two weeks and covered not only crack *b* but also the edges, *d*, *c*, *f* (Fig. 1), in the order named. Crack *b* received the largest amount of trash, edge *d* the next largest amount, while the edges *c* and *f* each received about an equal amount. The ants covering these cracks sometimes obtained the trash from one place on the island and sometimes from another; thus all the trips of the same ant were not made along the same path. Not only so, but the same ant often went to the trash pile along one path and returned to the crack along another.

In this particular experiment the glass cover over *C* reached much nearer the edge of the well than is shown in the illustration, which was drawn from another experiment of the same kind. Indeed, it entirely covered the turkish toweling on the well side of the living chamber. As a result of this, when the ants began to cover the edge *d* with trash, it would fall down into the well. This continued for nearly three days and by that time the well contained quite a collection of bread crumbs, bits of wood, and the charred ends of matches. About the close of the third day, the ants stopped carrying heavier debris and began covering the edge *d* with fibers of cotton shredded from the layer of cotton upon which the nest rested. They continued to add cotton fibers for about a day, at the end of which time they recommenced adding wood and bread crumbs to the pile. This time, owing to the presence of the cotton fibers this coarser detritus remained where placed. I do not feel justified in attaching much significance to the fact that after the other detritus failed to remain on the crack the ants covered it with cotton fibers and then resumed carrying heavier materials; for the cotton was all brought from a side of the nest upon which there was no other detritus and it may have been that the ants happened to go to that side for detritus and, finding cotton in abundance, continued to return to that side for material.

While these one to four workers were busy covering the cracks surrounding compartment *C*, yet others were busy filling compartment *A* (Fig. 1) with trash. A large number of workers assisted in filling compartment *A*, hence it was not long before this compartment was almost completely filled with trash and the entrance *E* so reduced in size that it was necessary to enlarge the opening whenever occasion arose to carry large pieces of captured food to those within the nest.

On an adjacent island I had a colony of the same species of ants in which there were no fertile females. In the early part of May these neuters began to lay eggs. Immediately compartment *A* was filled with trash.

Since the glass covering compartment *A* is colorless we must look upon the detritus placed there as trash heaped about the entrance to the nest. That the formation of such a trash pile about the opening of the nest is a common breeding habit or instinct is evidenced by the fact that in nature trash piles are found about the openings of many of the nests of this species. In the early spring I have frequently noticed such trash piles in the process of formation. They are composed partly of dirt brought from within the nest and partly of trash gathered from the surrounding territory. In a region where this species of ants is common, a careful search in the early spring is certain to reveal several such trash piles in the process of construction. In almost every case observed by me, a few ants were busy collecting trash from the outside and dropping it about the nest opening, while a larger number of ants were bringing dirt from the interior and heaping it about the same opening. It would then be illogical to consider the trash stored away in compartment *A* as anything more than the homologue of the trash pile that this species frequently builds about the entrance to its normal nests.

To me it does not seem logical to group the four piles of trash covering crack *b* and the edges *c*, *d*, *f* (Fig. 1) in the same category, for neither of these trash piles surrounded an entrance into the nest: *b*, the nearest of these piles, was at least seven inches from *E*, which was the only opening into the nest. Since this colony and its immediate ancestors had lived for several generations under the paving stones mentioned it is unlikely that either

they or their immediate ancestors had ever experienced a nest with a crack leading into the brood chamber.

To make sure that the covering of the cracks mentioned above was not a mere coincidence, I removed gently the trash that was covering crack *b*. In less than an hour a few ants were busy covering it. At intervals of about a week, this experiment was repeated twelve times; always yielding the same results. Usually one or two ants did the covering; at no one time have I seen more than four thus occupied.

That the ants were bent not on covering just any cracks that entered the nest, but only the cracks that affected the brood chamber is evidenced by the fact that, although these experiments covered a period of eight months, at no time did the ants cover the crack *a* or the free edges of the glass covers of compartments *A* and *B* (Fig. 1). Furthermore ants bearing trash would frequently cross the edges of the covers to *A* and *B* and even the crack *a* and pass on and deposit their burdens on the crack *b* or the edges *c*, *d*, or *f*. There is yet another evidence of this statement.

If this behavior is for the purpose of covering cracks the existence of which alters the conditions in the brood chamber, a crack crossing the brood chamber should produce a greater disturbance and hence should be covered by the ants first. Therefore I divided the cover to the living chamber *C* into two equal parts which were so adjusted as to leave between them a transverse crack *e* (Fig. 2) wide enough to make quite an opening yet too narrow to permit the passage of ants to and fro. Whenever this was done, and it was repeated over a dozen times, the first crack to be covered was *e*, and after *e*, *b* (Fig. 2). In each case only a few ants covered the cracks. Usually only one or two were thus employed; at no one time were over six thus occupied. Remember that that nest contained at least two hundred workers.

Whenever the trash was removed from crack *e* (Fig. 2), were it done ever so gently, the induced restlessness of the ants within the nest indicated that they were much disturbed. If I gently breathed against the uncovered crack, the ants within rushed about in all directions as though panic stricken. To one who

has watched this ant (*Formica fusca* var. *subsericea*) when outside the nest continue its work even when a breeze was blowing, the pronounced agitation caused by such a slight draught is sure to appear striking. It is, however, in harmony with observations, recorded in a former paper, upon the effect of sound and light

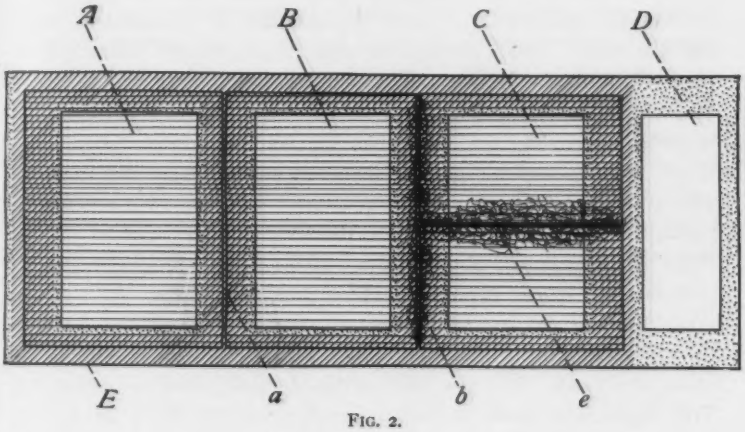


FIG. 2.

upon ants. It seems that the same lights and sounds and draughts which scarcely call forth any response when encountered without the nest, induce quite vigorous responses when encountered within the nest. In other words how an ant responds to a certain stimulus often depends upon whether the ant is within or without the nest.

What is the cause of this crack-covering behavior? Three possible solutions suggest themselves: first, the covering of the crack is a reflex activity induced by draughts through the cracks affecting the ants within the nest; second, it is a reflex response caused by odors that emerge through the crack and stimulate passing ants to cover it with trash; third, it is a response due to certain ants grasping the fact that the crack needed to be closed and then proceeding to cover it. The last supposition predicates to them what Hobhouse would call a practical judgment.

If the first assumption be true, then all of the ants within the brood chamber should have been stimulated to do the same

thing. Such was not the case; for although there were about two hundred workers within the nest, never more than six were seen covering the crack at any one time. Sometimes it took the few ants employed several days to cover the cracks in a satisfactory manner; yet in no case did a larger number participate.

To test the second assumption two different kinds of experiments were performed. I shall first describe an experiment of the first kind. When all the ants were resting quietly within the nest the trash was gently removed from crack *e* (Fig. 2). The ants within the nest immediately became restless. Two of them mounted the ceiling and examined the crack carefully. About fifteen minutes later an ant was observed moving back and forth on top of the nest. On coming in contact with crack *e* it paused momentarily, examined the crack carefully and then passed on. After it had roamed about the island for a few minutes, I imprisoned it. About five minutes later another ant from the nest walked across the top of the nest to crack *e*. After examining the crack carefully with its antennæ, the ant began to cover the crack with trash. In a few minutes this ant was joined by a third. This experiment shows that all ants that cross the crack are not stimulated to do the same thing.

The second type of experiment was quite unlike this. As before, the trash was gently removed from crack *e* producing the same restlessness within. The cover was then removed from compartment *B* (Fig. 1) and several worker ants transferred to a beaker. The cover was then replaced on *B*.

One of the captured ants, after having been marked with a characteristic color was placed near the middle of the top of compartment *C* and covered with a transparent white glass cone eight centimeters in diameter. At first the ant would make vigorous efforts to escape from the cone. It would meander over the top of the nest, go repeatedly round and round the circumference of the base of the cone and sometimes mount its sides. In these random movements the ant would of necessity cross that crack many times. After more or less of this fruitless activity, the ant would become quieter. It would then move more leisurely about, examining the crack at frequent intervals or else it would rest over the crack or a short distance therefrom and quietly preen its

antennæ. When this condition of calm had been attained, which usually required about five minutes, the glass cone was quietly removed. Thus the confining cone was not removed until the ant had recovered from the excitement caused by the handling.[1] Now if contact with crack *e* or the odors ascending through it will induce ants reflexly to cover it with trash, then, when free to roam at large, the ant should have proceeded to cover the crack with trash. This was tried with twelve different ants, but in no case did the ant cover the crack with trash. In all cases the ant carefully examined the crack and in one case it tried to force its way through the crack into the nest. Sooner or later the ant would begin to roam about over the top of the nest and in some cases over the Lubbock island as well. After awhile it would enter the nest. Some ants entered the nest within a minute after the confining cone was removed, one spent two hours finding its way home, one became lost and the majority took less than three minutes to find the entrance to the nest. About an hour after the beginning of the experiment an unmarked worker from the nest began to cover the crack with trash, and by the close of the third day it had covered cracks *e* and *b* in the usual manner (Fig. 2).

Once or twice a week for several months I continued to remove the trash from crack *e*, soon after it had been completely covered. Each time the crack was recovered. On June 28, however, the ants not only covered the top of *e* with trash, but beneath *e*, on the inside, they built up a wall of detritus, through which a tunnel connected the two halves of compartment *C*. The outside cover was composed of a heterogeneous mass of coarse particles of various kinds and a few cotton fibers; the partition constructed on the inside consisted of a felted mass of cotton fibers and fine crumbs of bread. Nine times I destroyed this inner portion; each time it was reconstructed by the ants out of the same kind of material. The tunnel through the partition was sometimes located in one position and sometimes in another. The different partitions varied in width from one fourth to three

[1] Experiments recorded in my paper on "The Homing of Ants" show that the handling of ants to mark them with water colors does not alter their physiological attunement.

fourths of an inch; the tunnels through the partition varied in width from one half an inch to one half the length of the partition. The ants glued the partition to the roof in such a manner that no matter how wide the tunnel, the felted roof always completely closed the crack.

After the ants had begun to close the crack with the wall built up within from the floor, the crack *e* was thereafter only imperfectly covered with trash; furthermore, instead of covering the edges *b*, *c*, *d* and *f* with trash, the same result was obtained by chinking from inside the space between the glass cover and the top of the walls of the brood chamber with material similar to that with which they constructed the inner partition.

To see if all colonies of *Formica fusca* var. *subsericea* Say would behave in the same way in the presence of a crack across their brood chamber, a crack was made in the top of the brood chamber of each of four nests of this species. These nests were obtained from the field and housed in Janet nests for this special purpose. In two cases the ants with their young deserted the chamber over which I had placed a crack and migrated to the nest of another colony of the same species.[1] In one case the ants with their young migrated from the compartment over which I had placed a crack into another compartment of the same nest. I forced them back into chamber *C* by substituting a piece of colorless glass for the orange glass with which the chamber into which they had migrated was covered. They and their young remained thereafter in chamber *C* for six weeks without doing anything that tended to close up that crack. In the fourth case the ants with their young retreated from brood chamber *C*, over which I had placed a crack, into chamber *B*. I then placed a crack across chamber *B* and a complete cover over chamber *C*. At once the ants covered the crack with trash but no partition was constructed upon the inside.

It is convenient to group the responses of animals living in colonies into class responses and individualistic responses. A class response is a stereotyped response which would be made by any and each member of a group when confronted with similar

[1] The colony into which each of these colonies migrated contained, before the arrival of the emigrants, no fertile females.

stimuli. When, under identical conditions, one member of a group responds to a stimulus in one way and yet other members respond to a similar stimulus in a different manner the response would be individualistic. When an animal faces a situation for which it has no class response and yet almost immediately makes an individualistic response which overcomes the difficulty, it has formed what Hobhouse calls a practical judgment.

It seems to me that this is what the ants did in the case recorded here. When a crack was made into their brood chamber they were face to face with a situation for which they had no class response. After awhile one to a few individuals made individualistic responses which resulted in the closing of the crack. To those few ants the disturbance in the brood chamber had been associated with the unclosed crack. To them the crack had acquired a meaning. It had become a crack-to-be-closed and they proceeded to close it.

It is not claimed that the construction of a trash pile of heterogeneous material, nor even the building of a felted partition out of special materials, indicates the formation of a practical judgment; for the forming of a trash pile by ants is, and the modeling of a partition may be, an instinctive action. But the utilization of these instinctive activities, without a preliminary period of experimentation, to meet adequately conditions for which the ants had no stereotyped response is what warrants the assumption that they form practical judgments.

It seems to me that in constructing the partial bridge, in removing the guards from the entrance and plugging it with cotton, and in closing the crack to the brood chamber, at first with trash piled on the outside and later with a wall built up from within, the ants have responded to stimuli, not as ends in themselves, but rather as means to ends. This would lift the act out of the realm of instinctive behavior into that of the practical judgment.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, August 17, 1907.

EXPLANATION OF FIGURES.

Each represents a diagram of the top of a Janet nest: *A*, *B*, *C* are brood chambers; *D* is the water well; *E* is the entrance to the nest; *a*, *b* are cracks between the glass covers; *c*, *d*, *f* are edges of the glass cover of chamber *C*; *e* is a crack across the top of chamber *C*; the oblique shading represents the turkish toweling; the horizontal shading represents the glass covers.

# THE CAUSATION OF MATURATION IN THE EGGS OF LIMPETS BY CHEMICAL MEANS.[1]

JULIAN MAST WOLFSOHN.

In his experiments on the maturation of the eggs of the starfish (*Asterias forbesii*)[2] Dr. Loeb found that the eggs when removed from the ovaries of the animal are in most cases immature, but that if they come in contact with sea water, during the breeding season they begin to maturate. The immature state is characterized by a large, plainly-visible nucleus, which, during maturation, becomes invisible. This process of maturation is completed in from one to two hours after the eggs are removed from the ovaries and placed in sea water. Only when the process of maturation is completed is it possible to fertilize the eggs with sperm.

Experimentation on the maturation of these eggs showed that the chemical conditions necessary to cause or accelerate the maturation processes are, that there must be present in the sea water two substances, free oxygen and hydroxyl ions of a certain concentration. Dr. Loeb found further that if, upon becoming mature the eggs were not caused to develop by the addition of sperm, then they died very rapidly. The change in the appearance of the egg after death is very marked; the living egg having a light yellow color, after death it becomes black; and where in the living egg the protoplasm is homogeneous and somewhat transparent, in the dead egg it becomes granular and opaque. Thus it was found that in a culture that had been standing for twenty-four hours, all the eggs that had remained immature were alive, and all those that had matured were dead. This shows that the mature eggs of the starfish die in the course of a few hours, while under exactly the same conditions the immature eggs remain alive.

When the maturation is prevented artificially through lack of

[1] From the Herzstein Research Laboratory of the University of California.

[2] BIOLOGICAL BULLETIN, Vol. 3, No. 6, Nov., 1902.

oxygen or by the addition of either acid or potassium cyanide to the sea water the eggs remain alive a considerable period of time. The eggs in which maturation has already begun, or has just been completed, are also saved from rapid death by these means.[1]

Later, he found that the eggs of a mollusc (*Lottia gigantea*) when removed from the ovary were always immature and would remain so even if they were kept in water for two days. Such eggs could not be fertilized by sperm. But if they were first treated with a mixture of 50 c.c. sea water and 1 c.c. 1/10 *n* NaHO for from four to five hours maturation took place with the result that over 75 per cent. of the eggs would develop into larvæ in normal sea water after the addition of sperm. He found also that treatment with sea water containing benzol would cause the eggs to become mature very rapiply.[2]

As in the case of the starfish egg the maturation processes in the eggs of *Lottia*, induced by alkali or benzol can be prevented by lack of oxygen, or by either the addition of acid or potassium cyanide to the sea water.

The fact that the immature eggs of the starfish and especially those of *Lottia* could be caused to become mature in this way, suggested experimentation upon the other limpets found at Pacific Grove. Four varieties of *Acmæa* were used by the writer. *Acmæa patina*, *pelta*, *persona* and *scabra*. The eggs of the *Acmæa* studied are all immature when removed from the ovaries. They look very much like those of *Lottia* (Fig. 1) and are characterized by a greenish color, a very irregular outline, and a transparent membrane — a chorion which conforms to the shape of the egg. In maturing the chorion disappears and the egg becomes perfectly spherical in shape and rather more opaque, Fig. 2. Only when this maturation process is completed is it possible to fertilize the egg by the addition of sperm. The eggs of *Acmæa* remain immature in sea water for two or three days and only occasionally does one find any mature eggs in the culture that has stood for any length of time.

To determine, whether by increasing the alkalinity of the sea

[1] These experiments have recently been repeated and confirmed by A.P. Matthews, *Am. Journal of Physiology*, 1907.

[2] *Univ. of Cal. Publications*, Nov. 17, 1905, Vol. 3, No. 1, pp. 1–8.

water the eggs would become mature, I subjected the eggs to the following solutions: 50 c.c. sea water, plus 0.4 c.c.; 0.7 c.c.; 1.0 c.c.; 1.5 c.c. 1/10 *n* NaHO for 1, 1.5, 2 and 3 hours. After each period the eggs were transferred to normal sea water

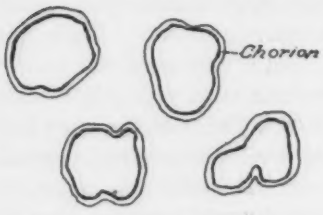


FIG. 1.—Immature eggs.

FIG. 2.—Mature eggs.

and sperm added about fifteen minutes later. At the end of two hours, maturation in most of the cases, was completed. Eighteen hours later the following results were obtained:

TABLE I.

| Solution. | Length of Treatment with Alkaline Sea Water. | | | |
|---|---|---|---|---|
| | 1 Hour. | 1.5 Hours. | 2 Hours. | 3 Hours. |
| 50 c.c. sea water + .4 c.c. *n*/10 NaHO. | 10 % mature. 1 swimming larva. Some disintegrated eggs. | 15 % mature. All swimming. | 40 % mature. 10 % swimming. Rest disintegrating. | 40 % mature. 5 % swimming. Rest disintegrating. |
| 50 c.c. sea water + .7 c.c. *n*/10 NaHO. | 30 % mature. 15 % larvæ. Few disintegrating. | 50 % mature. 40 % swimming. | 70 % mature. and swimming. 40 % irregular. | 90 % mature and swimming. 10 % disintegrating. |
| 50 c.c. sea water + 1 c.c. *n*/10 NaHO. | 40 % mature. All swimming. | 90 % mature and swimming. Few disintegrating. | 90 % swimming. 40 % irregular. | 98 % mature and swimming. 10 % disintegrating. |
| 50 c.c. sea water + 1.5 *n*/10 NaHO. | All mature and swimming. Fine larvæ. | 98 % mature and swimming. Few disintegrating. | 95 % mature and swimming. 20 % irregular. Disintegration. | 95 % swimming. 5 % disintegrating. |

From the table we see that the greatest number of the eggs become mature and form larvæ when 1.5 c.c. *n*/10 NaHO are added to 50 c.c. sea water, and the eggs subjected to this mix-

ture for one hour. Practically all become mature and the larvæ resulting are excellent.

If, however, the eggs are under-exposed or over-exposed to the solutions they either break up or segment irregularly and it is not very long before these latter eggs too, break up, and disintegrate. The number of disintegrating eggs is smaller when the eggs are treated for one hour with the mixture of 50 c.c. sea water and 1.5 c.c. *n*/10 NaHO than with any of the other solutions used. When sperm was added to the eggs which had not been treated with alkaline sea water, not a single egg became mature or developed into a swimming larva.

Next I tried the effects of lack of oxygen on maturation. The alkaline sea water (50 c.c. sea water + 1.5 c.c. *n*/10 NaHO) was placed in a bottle and connected with the hydrogen generator, and a stream of hydrogen was passed through the solution for two hours. The eggs were then quickly introduced and left for one hour in the solution, with a good stream of hydrogen still passing through. The eggs were then transferred to normal sea water and sperm added. The following table shows the result:

TABLE II.

| Solution. | Treatment for 1 hr. |
|---|---|
| 50 c.c. sea water + 1.5 c.c. *n*/10 NaHO with oxygen. | All mature and swimming.<br>Few eggs disintegrating. |
| 50 c.c. sea water + 1.5 cc. *n*/10 NaHO without oxygen. | About half a dozen mature eggs.<br>Two larvæ.<br>No disintegration. |

This shows that, as in the case with *Lottia*, the alkali can only cause the maturation of the eggs of *Acmæa* if oxygen be present. If the eggs that had been kept from becoming mature by lack of oxygen were subsequently treated with akaline sea water in the presence of oxygen, practically all became mature, and would, upon the addition of sperm, develop into larvæ.

By stopping the oxidative processes in the eggs through the presence of potassium cyanide, maturation can also be inhibited. Thus, if to alkaline sea water a little potassium cyanide be added no maturation occurs.

TABLE III.

| Solution. | Treatment for 1 hr. |
|---|---|
| 50 c.c. sea water + 1.5 c.c. n/10 NaHO. | All mature and swimming. Little disintegration. |
| 50 c.c. sea water + 1.5 c.c. n/10 NaHO + 1.2 c.c. 1/10% KCN. | Not one mature egg, in whole culture. No eggs disintegrated. |

The processes which were accelerated by the presence of the alkali were completely inhibited by the potassium cyanide and it is especially to be noted that while disintegrated eggs were found in every case in the absence of potassium cyanide, no disintegrated eggs were found when potassium cyanide was present.

If the eggs that had been prevented from becoming mature through the presence of the potassium cyanide, were afterwards treated with alkaline sea water, containing no potassium cyanide, practically all became mature and formed larvæ when sperm was added. This shows further that no permanent injury is done to the eggs by treating them with KCN or by depriving them of oxygen for so short a period of time.

I next tried the effect of fat solvents, such as benzol, chloroform, ether and ethyl acetate, upon the immature eggs to see if by treatment with these substances maturation could be produced, and I found that in every case I got positive results. The method of procedure in general was as follows: 30 c.c. sea water were heated to 45° C. and a known quantity of the solvent was added and the mixture vigorously shaken to ensure complete solution. If the solution is not complete the eggs that come in contact with the droplets of the solute are immediately killed. The solution was then cooled down to 26° C., and the eggs introduced. At definite periods the eggs were removed to normal sea water and sperm added. Eighteen hours after the results obtained in Table IV were noted.

The best results were obtained when the eggs were exposed to the mixture for one minute; 85 per cent. of the eggs developing into larvæ upon the addition of sperm. If the eggs are exposed longer to the solution they become mature, but a large number segment irregularly. These irregularly segmenting eggs finally disintegrate before they reach the larval stage, or in the early larval stage. By this method of treatment the eggs become

mature much more rapidly than by the treatment with alkali. Usually from three fourths to one hour after treatment the eggs will be mature, whereas when the eggs are simply treated with the alkaline sea water maturation rarely takes place in less than two hours.

TABLE IV.

| Solution. | Treatment for | | | | |
|---|---|---|---|---|---|
| | ½ Minute. | 1 Minute. | 1.5 Minutes. | 2 Minutes. | 2.5 Minutes. |
| 30 c.c. sea water + 2 drops benzol. | 25 % mature. 20 % swimming. Good larvae. | 90 % mature. 85 % swimming. Good larvae. Little disintegration. | 95 % mature. 50 % swimming. More disintegration. | 90 % mature. 60 % swimming. Great many disintegrated eggs. | 90 % mature. 60% swimming. 30 % disintegrated. |

The maturation induced by the treatment with benzol is also an oxidative process, since it does not occur when the oxidations are prevented in the egg. The addition of 1 c.c. 1/10 per cent potassium cyanide to the sea water inhibits the maturation of the eggs treated with benzol, and it is especially noteworthy that not a trace of disintegration is present in the eggs that were over-exposed to the solution in the presence of potassium cyanide. With the other fat solvents I have not as yet been able to get such a large percentage to develop as the table below will show.

TABLE V.

| Solution. | Exposure. | | |
|---|---|---|---|
| | ¾ Minute. | 1.5 Minutes. | 2.5 Minutes. |
| 30 c.c. sea water + 2 drops chloroform. | 10 % swimming. | 20 % swimming. | 25 % swimming. Many eggs killed. |
| 30 cc. sea water + 4 drops *n*/10 ether. | No larvæ. | 5 % swimming. | 10 % swimming |
| 30 c.c. sea water + 6 c.c. *n*/2 ethyl acetate. | 20 % larvæ. | 40 % larvæ. | 50 % larvæ. |

These last experiments were not worked out as thoroughly as those with benzol on account of the lack of material, but we can readily see that all the solutions tried give positive results.

## Conclusions.

1. As in the case of *Lottia* so also in that of *Acmæa patina*, *pelta*, *persona* and *scabra*, the addition of a small quantity of alkali to the sea water will cause the eggs to become mature.

2. The presence of oxygen hastens the destruction of the mature unfertilized eggs of *Acmæa*.

3. Lack of oxygen or the inhibition of the oxidative processes in the egg by the addition of a little potassium cyanide to the sea water not only prevents maturation but prevents also the disintegration of the mature unfertilized egg.

4. The treatment of the immature eggs of *Acmæa* with fat solvents, such as benzol, chloroform, ether, and ethyl acetate will also cause them to become mature.

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